Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases

Interim guidance
17 January 2020

World Health Organization

1. Introduction

The purpose of this document is to provide interim guidance to laboratories and stakeholders involved in laboratory testing of patients who meet the definition of suspected case of pneumonia associated with a novel coronavirus identified in Wuhan, China (See: Surveillance case definitions for human infection with novel coronavirus, Interim guidance).

Various existing WHO documents have been adapted for use in the drafting of this document, including WHO laboratory guidance for MERS-CoV (1-11). As information about the etiology, clinical manifestations and transmission of disease in the cluster of respiratory disease patients identified in Wuhan is evolving, WHO continues to monitor developments and will revise these recommendations as necessary.

The etiologic agent responsible for the cluster of pneumonia cases in Wuhan has been identified as a novel betacoronavirus, (in the same family as SARS-CoV and MERS-CoV) via next generation sequencing (NGS) from cultured virus or directly from samples received from several pneumonia patients. Electron microscopy revealed a virus with a characteristic crown morphology: a coronavirus. Working directly from sequence information, the team developed a series of genetic amplification (PCR) assays used by laboratories associated with the China CDC to detect several dozen cases as of today.

Full genome sequence data from the viruses have been shared officially with WHO and on the GISAID platform (https://www.gisaid.org/) and can inform the development of specific diagnostic tests for this emergent coronavirus. It is expected that validated PCR tests will become available soon. Until that time, the goals of diagnostic testing are to detect conventional causes of pneumonia early, to support disease control activities, and to work with reference laboratories that can perform pan coronavirus detection and directed sequencing.

2. Suspected case definition

For case definition see: WHO Surveillance case definitions for human infection with novel coronavirus.

3. Specimen collection and shipment

Rapid collection and testing of appropriate specimens from suspected cases is a priority and should be guided by a laboratory expert. As extensive testing is still needed to confirm the 2019-nCoV and the role of mixed infection has not been verified, multiple tests may need to be performed and sampling sufficient clinical material is recommended. Local guidelines should be followed regarding patient or guardian's informed consent for specimen collection, testing and potentially future research.

Assure SOPs are available, and the appropriate staff is trained and available for appropriate collection, specimen storage, packaging and transport. There is still limited information on the risk posed by the reported coronavirus found in Wuhan, but it would appear samples prepared for molecular testing could be handled as would samples of suspected human influenza (2, 7-9). Attempts to culture the virus may require heightened biosafety control measures.

Samples to be collected (see Table 1 for details on sample collection and storage):

1. Respiratory material* (nasopharyngeal and oropharyngeal swab in ambulatory patients and sputum (if produced) and/or endotracheal aspirate or bronchoalveolar lavage in patients with more severe respiratory disease)
2. Serum for serological testing, acute sample and convalescent sample (this is additional to respiratory materials and can support the identification of the true agent, once serologic assay is available)

*Modifiable with information on whether upper or lower respiratory material is better for coronavirus detection.

A single negative test result, particularly if this is from an upper respiratory tract specimen, does not exclude infection. Repeat sampling and testing, lower respiratory specimen is strongly recommended in severe or progressive disease. A positive alternate pathogen does not necessarily rule out either, as little is yet known about the role of coinfections.

Reference 2, 3, 7

Table 1. Specimens to be collected from symptomatic patients
Guidance on specimen collection (adapted from reference 5)

Specimen type	Collection materials	Transport to laboratory	Storage till testing	Comment
Nasopharyngeal and oropharyngeal swab	Dacron or polyester flocked swabs*	4 °C	≤5 days: 4 °C >5 days: -70 °C	The nasopharyngeal and oropharyngeal swabs should be placed in the same tube to increase the viral load.
Bronchoalveolar lavage	sterile container *	4 °C	≤48 hours: 4 °C >48 hours: –70 °C	There may be some dilution of pathogen, but still a worthwhile specimen
(Endo)tracheal aspirate, nasopharyngeal aspirate or nasal wash	sterile container *	4 °C	≤48 hours: 4 °C >48 hours: –70 °C	
Sputum	sterile container	4 °C	≤48 hours: 4 °C >48 hours: –70 °C	Ensure the material is from the lower respiratory tract
Tissue from biopsy or autopsy including from lung	sterile container with saline	4 °C	≤24 hours: 4 °C >24 hours: –70 °C	
Serum (2 samples acute and convalescent possibly 2-4 weeks after acute phase)	Serum separator tubes (adults: collect 3-5 ml whole blood)	4 °C	≤5 days: 4 °C >5 days: –70 °C	Collect paired samples: • acute – first week of illness • convalescent – 2 to 3 weeks later
Whole blood	collection tube	4 °C	≤5 days: 4 °C >5 days: –70 °C	For antigen detection particularly in the first week of illness
Urine	urine collection container	4 °C	≤5 days: 4 °C >5 days: –70 °C	

*For transport of samples for viral detection, use VTM (viral transport medium) containing antifungal and antibiotic supplements. For bacterial or fungal culture, transport dry or in a very small amount of sterile water. Avoid repeated freezing and thawing of specimens.

Aside from specific collection materials indicated in the table also assure other materials and equipment are available: e.g. transport containers and specimen collection bags and packaging, coolers and cold packs or dry ice, sterile blood-drawing equipment (e.g. needles, syringes and tubes), labels and permanent markers, PPE, materials for decontamination of surfaces.

Safety procedures during sample collection and transport

All specimens collected for laboratory investigations should be regarded as potentially infectious, and HCWs who collect, or transport clinical specimens should adhere rigorously to infection prevention and control guidelines and national or international regulations for the transport of dangerous goods (infectious substances) to minimize the possibility of exposure to pathogens (14). Implement the appropriate infection prevention and control precautions, guidance on IPC for the 2019-nCoV has been drafted (11).

Assure good communication with the laboratory and provide needed information

To assure proper and fast processing of samples and to assure adequate biosafety measures in the laboratory, communication and information sharing is essential. Be sure you have alerted the laboratory of the urgency and situation before sending the sample. Also assure that specimens are correctly labelled, and diagnostic request forms are filled out properly and clinical information is provided (see box: information to be recorded).

Information to be recorded:

- Patient information – name, date of birth, sex and residential address, unique identification number, other useful information (e.g. patient hospital number, surveillance identification number, name of hospital, hospital address, room number, physicians' name and contact information, name and address for report recipient),
- Date and time of sample collection,
- Anatomical site and location of specimen collection,
- Tests requested,
- Clinical symptoms and relevant patient history (including vaccination and antimicrobial therapies received, epidemiological information, risk factors).

Infection prevention measurements during sample collection for a novel coronavirus (route of transmission unknown but suspected to be respiratory)

Ensure that Health Care workers (HCWs) who collect specimens follow the following guideline and use the adequate PPE: Infection prevention and control during health care when novel coronavirus (nCoV) infection is suspected, interim

guidance, January 2020 (11) and other IPC guidance (10, 15-17).

Ensure that HCWs performing **aerosol-generating procedures (**i.e. aspiration or open suctioning of respiratory tract specimens, intubation, cardiopulmonary resuscitation, bronchoscopy) use additional precautions (for details see detailed guidelines mentioned above):

- Respirators (NIOSH-certified N95, EU FFP2 or equivalent, or higher level of protection). When putting on a disposable particulate respirator, always check the seal/fitness. Be aware that the presence of facial hair (e.g. beard) may prevent a proper respirator fit for the wearer. In some countries, a powered air-purifying respirator (PAPR) is utilized instead of a respirator.
- Eye protection (i.e. goggles or a face shield).
- Clean, long-sleeved gown and gloves. If gowns are not fluid resistant, a waterproof apron should be used for procedures where it is expected that fluid might penetrate the gown

• Perform procedures in an adequately ventilated room: at a minimum natural ventilation with at least 160l/s/patient air flow, or negative pressure rooms with at least 12 air changes per hour and controlled direction of air flow when using mechanical ventilation
• Limit the number of persons present in the room to the minimum required for the patient's care and support; and
• Follow WHO guidance for steps of donning and doffing PPE. Perform hand hygiene before and after contact with the patient and his or her surroundings and after PPE removal.
• Waste management and decontamination procedures: Ensure that all materials used is disposed appropriately. Disinfection of work areas and decontamination of possible spills of blood or infectious body fluids should follow validated procedures, usually with chlorine-based solutions.

Specifics for transport of samples to laboratory:
• Ensure that personnel who transport specimens are trained in safe handling practices and spill decontamination procedures.
• Follow the requirements in the national or international regulations for the transport of dangerous goods (infectious substances) as applicable (14).
• Deliver all specimens by hand whenever possible. Do not use pneumatic-tube systems to transport specimens.
• State the full name, date of birth of the suspected case clearly on the accompanying request form. Notify the laboratory as soon as possible that the specimen is being transported.

Packaging and shipment to another laboratory
Transport of specimens within national borders should comply with applicable national regulations. International Transport Regulations. Novel coronavirus specimens should follow the UN Model Regulations, and any other applicable regulations depending on the mode of transport being used. More information may be found in the WHO Guidance on regulations for the Transport of Infectious Substances 2019-2020 (Applicable as from 1 January 2019) (14). A summary on transport of infectious substances can also be found in Toolbox 4 of the Managing epidemics handbook (1).

4.Effective usage of Global Laboratory Networking

be sought from a reference laboratory that can deploy additional or confirmatory assays.

Timely and accurate laboratory testing of specimens from cases under investigation is an essential part of the management of emerging infections. All countries should have access to reliable testing, either nationally or internationally, in laboratories willing to perform primary detection or confirmatory testing, and novel pathogen detection. WHO is currently working closely with collaborating centres and experts to assure diagnostics will be developed and validated promptly. WHO can assist Member States to access testing internationally should the need arise.

5.Testing of 2019-nCoV in reference laboratories

2019-nCoV testing for patients that meet the suspected case definition

Patients that meet the case definition for suspected 2019-nCoV should be screened for the virus with PCR (details below). If case management requires, screen also for other common causes of respiratory illness according to local guidelines (1,5,7). As coinfections can occur, all patients that meet the case definition should be tested for 2019-nCoV regardless of whether a conventional respiratory pathogen is found. If testing does not occur in an expert/reference laboratory it is encouraged to send the sample for confirmation to a regional, national or international reference laboratory with pan-coronavirus or specific 2019-nCoV detection capacity. WHO can assist Member States to identify laboratories able to provide this support.

Nucleic acid amplification tests for 2019-nCoV

As sequence information from the 2019-nCoV has recently been made available, PCR assays can be designed to detect these sequences. PCR assay design optimization can be a complicated process, and a useful option is to contact the experienced laboratories publicizing their assays and request access to their assay chemistries.

Laboratories may desire to use a pan-coronavirus assay for amplification followed by sequencing of amplicons from non-conserved regions for characterization and confirmation. The importance of the need for confirmation of results of testing with pan-coronavirus primers is underscored by the fact that four human coronaviruses (HcoVs) are endemic globally: HCoV-229E, HCoV-NL63, HCoV-HKU1 as well as HCoV-OC43. The latter two are betacoronaviruses. Two other betacoronaviruses that cause zoonotic infection in humans are MERS-CoV, acquired by contact with dromedary camels and SARS arising from civets and cave-dwelling horseshoe bats.

Alternatively, amplification and detection of 2019-nCoV specific sequences can be diagnostic without the necessity for further sequencing. In the case of surprising findings or for less-experienced laboratories, external assistance should

Once specific NAAT assays are developed and validated, confirmation of cases of the novel virus infection will be based on specific detection of unique sequences of viral nucleic acid by reverse-transcriptase polymerase chain

reaction (RT-PCR). Alternative NAAT techniques with advantages of greater speed or simplicity of use may also become available.

Serological testing

Serological testing may be useful to confirm immunologic response to a pathogen from a specific viral group, e.g. coronavirus. Best results from serologic testing requires the collection of paired serum samples (in the acute and convalescent phase) from cases under investigation.

Sequencing in outbreaks

Sequence data can provide valuable information for understanding the origin of a virus and how it is spreading. WHO has published a Draft code of conduct for the handling of Genetic Sequence Data related to outbreaks (see https://www.who.int/blueprint/what/norms-standards/GSDDraftCodeConduct_forpublicconsultation-v1.pdf?ua=1). For situations where data providers seek retention of ownership of their data, models with data access agreements (such as GISAID) have been used to facilitate rapid sharing of genetic sequence data. Laboratories are encouraged to share sequence data with WHO and the scientific community to assist in the rapid development and distribution of diagnostic assays in at risk countries. The uploading and public access to sequence data should occur prior to journal publication. Medical journals should ensure that their policies actively support pre-publication sharing of pathogen genetic sequence data together with appropriate acknowledgement. WHO can assist Member States to identify laboratories able to provide support and advise them on the management of sequence data related to an outbreak.

Specifics for biosafety practices in the laboratory

Ensure that health laboratories adhere to appropriate biosafety practices. Any testing on clinical specimens from patient meeting the case definition should be performed in appropriately equipped laboratories by staff trained in the relevant technical and safety procedures. National guidelines on the laboratory biosafety should be followed in all circumstances. General information on laboratory biosafety guidelines, see the WHO Laboratory Biosafety Manual, 3rd edition (8). There is still limited information on the risk posed by the reported coronavirus found in Wuhan, but it would appear samples prepared for molecular testing could be handled as would samples of suspected human influenza (2, 7-9).

It is recommended that all manipulations in laboratory settings of samples originating from suspected or confirmed cases of novel coronaviruses can be conducted according to WHO recommendations available at: https://www.who.int/csr/disease/coronavirus_infections/Biosafety_InterimRecommendations_NovelCoronavirus2012_31Oct12.pdf?ua=1 Information on biosafety for SARS, a Betacoronavirus that can cause severe respiratory disease can be consulted at https://www.who.int/csr/sars/biosafety2003_04_25/en/. and other guidance.

Table 2. Tests to be performed in expert laboratories for patients meeting the case definition

Test	Type of sample	Comments
In laboratories that have validated broad coronavirus RT-PCR assays it is advised to check the primers against the published 2019-nCoV sequence and check if primers are overlapping and have the capacity to detect the 2019-nCoV. On a positive results sequencing should be performed to determine the precise virus detected (e.g. on an amplicon of a non-conserved region).	Respiratory sample	Collect on presentation. Done by an expert laboratory.
NAAT for 2019n-CoV when it becomes available (assays currently under validation)	Respiratory sample	Collect on presentation. Done by an expert laboratory until validation has been finalized.
Whole genome sequencing	Respiratory sample	Collect on presentation. Done by an expert laboratory.
Serology, broad corona virus serology on paired samples if available.	Serum	Paired samples necessary for confirmation, the first sample collected in week 1 of illness and the second collected 3-4 weeks later. If a single serum sample can be collected, collect at least 3 weeks after onset of symptoms. Done by expert laboratory until more information on performance of available assays.

6. Reporting of cases and test results

Laboratories should follow national reporting requirements, but in general, suspected cases should be reported to relevant public health authorities as soon as the laboratory receives a specimen, even before any testing is performed. All

test results, whether positive or negative, should likewise be immediately reported to national authorities. If the infection becomes widespread, laboratories should notify public health authorities immediately of each new confirmed case or positive screening test if there will be a delay in confirmatory testing. Laboratories should also periodically report the number of negative test results to public health.

States Parties to the IHR are reminded of their obligations to share with WHO relevant public health information for events for which they notified WHO, using the decision instrument in Annex 1 of the IHR (2005) (18).

Detection of a possible human case of emerging pathogen causing severe acute respiratory disease should immediately be notified to local, subnational and national public health authorities. This will allow these authorities to make immediate decisions about launching the investigation and the extent of response measures. Detection of such a case should be used to trigger notification of traditional and non-traditional health providers, hospitals and outpatient. Facilities, and community leaders in the area where the case patients lived or travelled, as part of active case-finding efforts. In line with the International Health Regulations (IHR) (2005), the national health authority must notify WHO within 24 hours of all events that may constitute a public health emergency of international concern according to defined criteria. The IHR decision instrument should be used to determine whether an event is to be notified to WHO. Further guidance on the use of the IHR decision instrument, including examples of its application, is available. The national animal health authority must notify OIE of certain animal diseases detected on its territory. OIE focal points should be contacted for further details.

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